

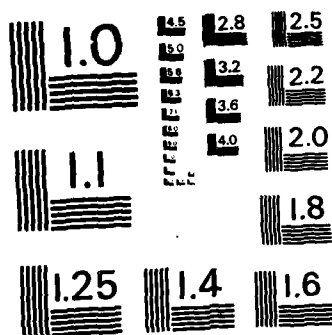
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Unilateral mechanical lesions of the anterior hypothalamic/pre-optic (AH/PO) region of the rat were found to produce immediate pyrexia. The pyrexia was generated by the coordinated activation of heat gain and heat retention effectors. Its magnitude was not strongly affected by ambient temperature, and the plateau level of pyrexia was well defended in the face of forced perturbations of core temperature. Pyrexia could be prevented and reversed by		

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the prostaglandin synthesis inhibitor, indomethacin. Intraventricular injection of fresh blood or serum derived from blood which had been incubated under sterile conditions at 37°C for from 2 hours to 21 days produced pyrexia in cats. Pretreatment of the cats with indomethacin prevented the pyrexia produced by the serums but including indomethacin in the incubating blood did not. These results indicate that prostaglandins are importantly involved in the production of pyrexia by AH/PO trauma and by intraventricular bleeding. Studies of the central nervous system site of action of prostaglandins in the production of pyrexia using a microinjection "mapping" method showed that the AH/PO region is the sole site of action in the upper portion of the rat brain. Similar studies of the posterior portion of the rat brain have revealed that there probably exists at least one lower brain stem site where prostaglandins can act to produce pyrexia. Injections of prostaglandins into the rat spinal subarachnoid space did not produce increases in core temperature.

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HYPERPYREXIA AND HEAD TRAUMA

FINAL REPORT

by

Thomas A. Rudy, Responsible Investigator

(For the period 1975-1983)

Supported by

Office of Naval Research
 Contract N00014-75-C-0939
 University of Wisconsin
 Madison, Wisconsin 53706

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I. Publications

A. Papers and book chapters

- (1) Williams, J. W., Rudy, T. A., Yaksh, T. L. and Viswanathan, C. T. An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. *Brain Research*, 120: 251-262 (1977).
- (2) Komiskey, H. L. and Rudy, T. A. Serotonergic influences on brainstem thermoregulatory pathways in the cat. *Brain Research*, 134: 297-315 (1977).
- (3) Rudy, T. A., Williams, J. and Yaksh, T. L. Antagonism by indomethacin of neurogenic hyperthermia produced by unilateral puncture of the anterior hypothalamic/preoptic region. *J. Physiol.*, 272: 721-736 (1977).
- (4) Rudy, T. A. and Yaksh, T. L. Hyperthermic effects of morphine: Set point manipulation by a direct spinal action. *Br. J. Pharmacol.*, 61: 91-96 (1977).
- (5) Rudy, T. A. and Yaksh, T. L. Elevation of the setpoint for thermoregulation by intrahypothalamic injection of carbamylcholine in the rhesus monkey. IN Cooper, K. E., Lomax, P. and Schonbaum, E. (eds.), Drugs, Biogenic Amines and Body Temperature, Karger, Basel, pp. 26-30 (1977).
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- (7) Ackerman, D. and Rudy, T. A. Thermoregulatory characteristics of neurogenic hyperthermia in the rat. *J. Physiol.*, 307: 59-70 (1980).
- (8) Rudy, T. A. Studies of fever associated with cerebral trauma and intracranial hemorrhage in experimental animals. IN Lipton, J. M. (ed.), Fever, Raven Press, New York, pp. 165-175 (1980).
- (9) Rudy, T. A. Pathogenesis of fever associated with cerebral trauma and intracranial hemorrhage. IN Cox, B., Lomax, P., Milton, A. S. and Schonbaum, E. (eds.), Thermoregulatory mechanisms and their therapeutic implications, Karger, Basel, pp. 75-81 (1980).
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- (11) LoPachin, R. and Rudy, T. A. The effect of intrathecal sympathomimetic agents on neural activity in the lumbar sympathetic chain of rats. *Brain Research*, 224: 195-198 (1981).

- (12) LoPachin, R. and Rudy, T. A. The thermoregulatory effects of noradrenaline, serotonin and carbachol injected into the rat spinal subarachnoid space. *J. Physiol.*, 333: 511-529 (1982).
- (13) LoPachin, R. and Rudy, T. A. Sites and mechanism of action for the effects of intrathecal noradrenaline on thermoregulation in the rat. *J. Physiol.*, 341: 527-544 (1983).
- (14) O'Rourke, S. T. and Rudy, T. A. Intracerebroventricular and preoptic injections of leukotrienes C₄, D₄ and E₄ in the rat: Lack of febrile effect. *Brain Research*, 295: 283-288 (1984, in press).

B. Abstracts and presentations

- (1) Williams, J. W. and Rudy, T. A. An extensive neuroanatomical mapping of the rat brain for sites which mediate prostaglandin-induced hyperthermia. *Neurosci. Abstracts*, 1: 417 (1975).
- (2) Rudy, T. A. and Komiskey, H. L. Functionally antagonistic thermoregulatory effects of 5-hydroxytryptamine within the anterior hypothalamus and preoptic area of the cat. *Fed. Proc.*, 35: 530 (1976).
- (3) Rudy, T. A., Williams, J. W. and Yaksh, T. L. Hyperthermia evoked by acute mechanical damage to the hypothalamus and its antagonism by indomethacin. *Neurosci. Abstracts*, 2: 731 (1976).
- (4) Rudy, T. A. Thermoregulatory effects of 5-hydroxytryptamine injected into the brainstem of the cat: Observation of two anatomically distinct sites of action. Paper read at the Third International Symposium on the Pharmacology of Thermoregulation, Banff, Canada, 1976.
- (5) Ackerman, D. and Rudy, T. A. The effect of ambient temperature on the hyperthermia evoked by acute mechanical damage to the hypothalamus. *Neurosci. Abstracts*, 3: 393 (1977).
- (6) LoPachin, R. and Rudy, T. A. The effects on body temperature of norepinephrine and 5-hydroxytryptamine injected into the rat spinal subarachnoid space. *Fed. Proc.*, 38: 756 (1979).
- (7) Rudy, T. A. Pathogenesis of fever associated with cerebral trauma and intracranial hemorrhage. Paper read at the Fourth International Symposium on the Pharmacology of Thermoregulation, Oxford, England, 1979.
- (8) Rudy, T. A. Studies of fever associated with cerebral trauma and intracranial hemorrhage in experimental animals. Paper read at the First International Symposium on Fever, Dallas, Texas, 1979.

- (9) LoPachin, R. and Rudy, T. A. Spinal cord monoaminergic and cholinergic mechanisms in thermoregulation. *Pharmacologist*, 22: 184 (1980).
- (10) Rudy, T. A. Enhanced pyrogenicity of decomposed versus fresh blood injected intraventricularly in the cat. *Fed. Proc.*, 39: 991 (1980).
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- (13) Rudy, T. A. and Gollman, H. M. The role of cyclooxygenase products in fever elicited by intraventricular injection of serum derived from aged blood. *Fed. Proc.*, 1984 (in press).

II. Personnel supported

This contract provided full or partial salary support for the following individuals:

Tony L. Yaksh (postdoctoral associate)
Jerome L. Westergaard (specialist)
Joseph C. Yeung (graduate student)
Bruce Cornwell (specialist)
Richard LoPachin (graduate student)
Patricia Koch (specialist)
Deborah Ackerman (graduate student)
Stephen T. O'Rourke (graduate student)
Harold Gollman (graduate student)

Contract funds were also used to compensate the following University of Wisconsin undergraduate students for part time clerical or laboratory work related to the goals of the contract:

Michael Cain
Karin Gast
Sung-Ping Huang
Jennifer Ondrajka
Robert Plant
Sandra White
Patricia Hick
Stanley Tam
Dennis Hu
Ronald Epperson
Diane Boszhardt
Vicki Post
Jackie Wilson
Domenick Garzone
Gordon Meyer
Patricia Duffy
Keith Zelhafer
Debra Finley
Debra Brown
Julia Simon
Roger Hess
Ron Paulson

III. Overview of Contract Accomplishments

The general goal of the work supported by this contract was to gain a better understanding of the pathophysiological basis of the pyrexias which sometimes follow cranio-cerebral trauma. A review of the clinical and experimental literature suggested that these "trauma-induced fevers" could be due to destruction of or injury to cerebral tissue, particularly that of the hypothalamus, and/or to the effect of blood entering the cerebral ventricles or subarachnoid spaces. A specific goal, therefore, was to attempt to develop animal models of trauma-induced fevers in which hyperthermia would be elicited by intentional lesioning of the hypothalamus or by introduction of blood into the cerebral ventricles. These models would then be used to study the fundamental mechanisms through which the pyrexias were produced, with a particular emphasis on the possible involvement of arachidonic acid metabolites (e.g., prostaglandins) and certain CNS neurotransmitters. The following narrative summarizes the results obtained from studies supported by the contract. Details can be obtained from the publications (appended) stemming from these studies.

A. Pyrexia produced by acute mechanical destruction of brain tissue (Papers #3 and #7).

Our first accomplishment was to develop a model in the rat of the acute pyrexia produced by rapidly developing injury to the anterior hypothalamic/preoptic (AH/PO) region of the brain. In this model, an 18 ga stainless steel guide tube was permanently implanted just above one side of the AH/PO region, and the tube was occluded with a solid stylet the same length as the guide. To produce a lesion, the stylet was removed and replaced with one 6 mm longer than the guide. Insertion of the longer stylet produced instantaneous destruction of the medial AH/PO region on one side. It was shown in a series of more than 80 rats that such lesioning produced with nearly 100% reliability a pyrexia which began immediately, reached its peak magnitude (mean peak magnitude = $+2.3^{\circ}\text{C}$) within 30 to 90 minutes and lasted 8 to 16 hours. The pyrexia was not a consequence of convulsive activity, increased motor activity or behavioral excitability. The fact that a unilateral injury could produce pyrexia suggested to us that at least some types of trauma-induced fevers did not depend upon a disinhibition of brain-stem structures caudal to the lesion which control thermogenesis and/or upon destruction of AH/PO neurons responsible for heat dissipation. The hypothalamus is bilaterally redundant, both anatomically and functionally. A lesion on one side only is therefore highly unlikely to result in a disinhibition of heat gain or a loss of the ability to dissipate heat.

It was also demonstrated that pretreatment of the rats with the prostaglandin synthesis inhibitor, indomethacin, would reduce the magnitude of the pyrexia in a dose dependent fashion. In fact, a dose of 15 mg/kg given 1 hour before lesioning virtually abolished the effect (88% reduction in the 6-hour Fever Index). It was also found that injection of indomethacin in a rat already made pyrexemic by lesioning caused body temperature to return rapidly to the pre-

lesioning level. As a result of other work supported by the contract (see below), we knew that prostaglandins injected into the AH/PO region of the rat elicit hyperthermia. It was also known that brain tissue can synthesize prostaglandins in response to injury. We therefore formulated the hypothesis that unilateral AH/PO injury caused pyrexia through the release of prostaglandins from the injured tissue. These prostaglandins were posited to act on surviving AH/PO tissue on the lesioned side and/or by diffusion to the intact AH/PO tissue on the side contralateral to the lesion.

The "disinhibition/loss of function" hypothesis has often been cited in the literature as a speculative explanation of trauma-induced hyperthermia. As indicated above, our data argue against the involvement of such a mechanism in at least some trauma-induced fevers. Another frequently mentioned explanation of trauma-induced fevers is the "neurogenic" hypothesis. In general terms, this hypothesis suggests that neurons in the tissue surrounding the lesion are rendered hyperirritable by non-specific processes such as mechanical displacement, edema or ischemia. This, in turn, was speculated to result in persistent activation of neuronal systems controlling heat gain, with a resultant rise in core temperature. It is noteworthy that our own hypothesis is not totally incompatible with the neurogenic hypothesis. Rather, we have simply substituted specific mediators (prostaglandins) for the non-specific effects associated with the neurogenic hypothesis. However, there are significant differences between the two hypotheses in regard to how the pyrexia is effected. Pyrexia produced by intracerebral injection of prostaglandins is generated and maintained by a coordinated modulation of thermogenic and heat retentive effectors, the magnitude of the hyperthermia is not strongly affected by variations in ambient temperature, and the elevated temperature is defended vigorously in the face of thermal stress. Effector coordination during a hyperthermia caused by the intractable stimulation of heat gain associated with the neurogenic hypothesis should be poor, the magnitude of the hyperthermia should be strongly affected by ambient temperature, and the elevated core temperature should not be well defended. We therefore carried out experiments to characterize the regulatory properties of pyrexias produced by unilateral mechanical AH/PO lesions in rats. In these studies we (a) observed the thermoregulatory effector activities which were responsible for generating the pyrexia, (b) observed the thermoregulatory reactions elicited by forced elevation and depression of core temperature during a pyrexia episode and (c) observed the effect of ambient temperature on pyrexia magnitude. The results indicated that the pyrexia produced by unilateral AH/PO puncture in the rat is generated through a coordinated effort of heat gain and heat retentive effectors, that the magnitude of the pyrexia is not strongly altered by changes in ambient temperature and that the plateau level of hyperthermia is defended vigorously. The results thus strongly support our contention that the lesion-induced pyrexias were mediated by prostaglandin release and not by a "neurogenic" mechanism involving continuous irritative activation of heat gain. A clinical implication of our findings is that prostaglandin synthesis inhibitors, given in adequate dosage, might well be of benefit in some cases of trauma-induced hyperthermia in humans.

B. Pyrexia produced by simulated intraventricular hemorrhage (Paper #6 and Abstracts #10 and # 13).

As indicated earlier, there is reason to believe that some pyrexias associated with cranio-cerebral trauma are dependent upon intraventricular bleeding rather than tissue damage for their genesis. Whole blood contains or releases during the clotting process several potentially pyrogenic substances and some known pyrogens, among them the prostaglandins. Prostaglandins of the "E" type injected intraventricularly or directly into the rostral hypothalamus or the preoptic region produce a rapidly developing pyrexia. The latter structures are situated close to the ependyma in the walls of the rostroventral aspect of the third cerebral ventricle. Thus, the third ventricular region offers a likely site of action if, in fact, blood can elicit a fever by acting from within the ventricular spaces.

Our first objective was to develop an animal model of pyrexia associated with ventricular bleeding. The second objective was to ascertain the approximate location of the reactive region of the ventricular system. To accomplish these objectives, we implanted cats with stainless steel guide cannulae with their tips situated in either the dorsal or ventral aspect of the third ventricle. After a 10 day recovery period, each animal was subjected to two experiments which were separated by minimum interval of 7 days. The first consisted of a control injection of 500 ul of a sterile, pyrogen-free artificial cerebrospinal fluid and the second, a similar injection of a fresh, non-anticoagulated sample of the animal's own venous blood. Four hours after the blood injection, the animals were killed and the distribution of blood in the ventricles determined.

The results showed that injections of blood often elicited a pyrexia response whereas the control injections did not. Because of misplacement of several guide cannulae and because of the development of scar tissue around some of the guide cannula tips, many of the blood injections distributed in ways other than had been intended. This was fortunate because it permitted us to determine that pyrexia developed after blood injection only when the blood had reached the rostroventral aspect of the third ventricle. Even large amounts of blood reaching other ventricular spaces failed to produce pyrexia. Thus, the AH/PO region is the likely site of action vis-a-vis the pyrogenic effect of intraventricular blood.

In subsequent work, it was shown that blood which had been incubated at 37°C under sterile conditions for periods up to 21 days was more pyrogenic than fresh blood. The idea to test aged blood was derived from clinical reports which suggested that fevers produced by breakthrough into a ventricle of the reliquified contents of old encapsulated hematomas were more intense than those produced by bleeding directly into a ventricle. We were also able to show that the particulate-free supernatant liquid derived by centrifugation of aged bloods was pyrogenic and that the pyrogenicity increased with incubation time. Supernatants derived from blood aged

15 days had the maximum pyrogenicity, but those derived from blood aged 6 days were nearly as active. Thus, some soluble pyrogen accumulates in the fluid portion of a clot as it ages under sterile conditions. Thus far, we have not carried out the experiments required to identify the pyrogen. However, we have evaluated the possible role of prostaglandins or other cyclo-oxygenase products. In these experiments, cats were given a systemic injection of indomethacin 1 hour prior to the intraventricular injection of supernatants which were known to be pyrogenic. These cats did not develop a pyrexia. However, supernatants derived from blood which had been incubated in the presence of a high concentration of indomethacin (25 ug/ml) were pyrogenic when injected in cats which had not received a systemic indomethacin injection. As it is known that indomethacin prevents prostaglandin synthesis but does not prevent the pyrogenic effect of preformed prostaglandins, the results of this study indicate that supernatant-induced pyrexia is not due to the presence of prostaglandins in the supernatant. However, the data also indicate that prostaglandins are somehow involved in the mediation of the pyrexia. Our working hypothesis is that a substance present in the supernatant acts on ependymal cells and/or on periventricular brain tissue to evoke the release of prostaglandins within the tissue and/or into the cerebrospinal fluid.

The possibility that the pyrogen in the supernatants may be endotoxin has been examined (Rudy, T. A., unpublished), but the results are inconclusive. Limulus Amebocyte Lysate (LAL) assay of supernatants derived from blood aged from 2 hours to 21 days showed that the supernatant volume that we now use routinely to produce pyrexia (200 μ l) contained from 36 to 59 pg of LAL-reactive material. This is a small amount but, as some endotoxins are strongly pyrogenic, the possibility that endotoxin might be responsible for the pyrogenicity of the supernatants cannot be dismissed. On the other hand, because of the large number and diverse nature of substances present in the supernatants, there is the possibility that the LAL-reactive material we detected is not endotoxin. Obviously, further experiments need to be done to assess the possible contribution of endotoxin. It is noteworthy, however, that our aged bloods and supernatants have been shown to be sterile and that our controls for preventing contamination of these products with endotoxin are rigid. Also, we have carried out experiments to ascertain at what point the LAL-reactive material enters the incubation system. We are convinced that, if the LAL-reactive material is endotoxin, that endotoxin was present in the cat's blood at the time it was drawn. That the blood of healthy animals can routinely contain small amounts of endotoxin is supported by several literature reports.

The possibility that the pyrogenic material present in blood and in supernatants derived from incubated blood may be 5-hydroxytryptamine or acetylcholine is discussed in later sections of this report.

c. Central nervous system site of pyrogenic action of prostaglandins (Paper #1).

As has been mentioned in preceding sections, we have hypothesized that some trauma-induced fevers are due to the action of prostaglandins on neuronal elements within the AH/PO region. At the time this contract began (1975), it was well established in the literature that prostaglandins E_1 and E_2 caused hyperthermia when injected intraventricularly. It had also been shown that injections of these prostaglandins directly into the AH/PO region produced a similar effect. However, it had not been demonstrated that the AH/PO region was the only CNS region where prostaglandins can act to cause hyperthermia; very few areas other than the AH/PO region had been examined. We therefore carried out a "mapping" study in which small amounts of PGE_1 (50-100 ug in 1 ul) were injected into various sites in the rat brain rostral to the medulla. The results are described in detail in Paper #1. In brief, it was found, after an examination of 272 sites, that all of the sites where PGE_1 injection produced a temperature rise were clustered in the AH/PO region. It was concluded that the AH/PO region is an important and probably the only supramedullary site of PGE_1 action in the rat brain. This finding forms the basis for our contention that prostaglandins released by hypothalamic trauma or by bleeding into the third ventricle cause hyperthermia by an action within the AH/PO region.

Subsequent to the completion of the study mentioned above, several reports appeared in the literature which strongly suggested the existence of an extrahypothalamic site of action for pyrogens/prostaglandins in the production of fever. The most convincing of these was the finding that total bilateral ablation of the AH/PO region in rhesus monkeys did not reduce the pyrogenic effect of systemically injected pyrogens or intraventricularly injected prostaglandins. We were also aware from a survey of the literature that pontine lesions in humans and in cats commonly resulted in hyperthermia, and it seemed possible that prostaglandins released from injured pontine tissue might be acting at a lower brain stem (or spinal cord) prostaglandin-responsive site to produce a temperature rise. We therefore undertook a study whose goal was to ascertain the location of the extrahypothalamic site of action, if, indeed, such a locus of action exists. We expect that two publications describing this work will be submitted this year.

The CNS region explored in this study ranged from the spinal cord (lumbar level) to the lower midbrain. Although the lower midbrain had been examined in the study described in Paper #1, the number of injection sites was small, and it was decided that a more extensive exploration of this area would be desirable. The spinal cord response to prostaglandins was examined first. The cord was considered a viable potential site of action because we had shown in ancillary experiments (Paper #4) that morphine injected into the spinal subarachnoid space could produce a large core temperature increase in the rat and that this increase was a regulated one (i.e., functionally equivalent to a true fever). This effect was likely due to a biasing within the cord of thermal input from skin, deep body or spinal cord thermodetector units such that a false "cold" signal was transmitted to supraspinal structures involved in

thermoregulation. We reasoned that it was possible that other substances, e.g., prostaglandins, might alter thermoregulation through a direct action on the cord. However, extensive studies in which a wide range of doses of PGE_1 and PGE_2 were injected via an indwelling spinal catheter into the lumbar, thoracic and cervical subarachnoid spaces revealed no consistent hyperthermic effect. The only consistent, dose-related effect produced by these prostaglandins was a core temperature fall elicited by lumbar level injections.

We next examined the reactivity of sites in the rat brain situated between the spinomedullary junction and frontal plane P-2 in the atlas of Pelligrino et al. We have thus far tested the ability of 100 ng of PGE_1 injected into 445 different sites in the posterior aspect of the rat brain to elicit a temperature rise. The vast majority of these sites were unreactive. However, injections into about 30 sites evoked a pyrexia response. Most of these reactive sites are clustered within two reasonably distinct regions which we prefer not to identify precisely until we publish the data later this year. We have focussed our attention on that region which responds most consistently. PGE_1 injections into this area produce a hyperthermic effect which is about half as large as that produced by injection of an equal amount of PGE_1 into the AH/PO region. The response begins immediately after injection and has the time course of an AH/PO injection. The response is reproducible within a given rat, both within a session and between sessions. Saline injections had no effect on body temperature. There is no indication the temperature rises are due to convulsions, enhanced motor activity or behavioral excitability.

The major impediment to pronouncing the above mentioned locus an authentic extrahypothalamic site of prostaglandin action is that it borders on a subarachnoid space. Injected PGE_1 might thus diffuse via the CSF to the AH/PO region and act there to effect a pyrexia response. Evidence against this possibility gained thus far is (a) the injection volume is small and the response begins immediately, (b) injections at numerous other loci bordering on subarachnoid spaces or on ventricular spaces have not produced pyrexia, (c) injections of 100 ng of PGE_1 in 1 μl directly into the fourth ventricle, aqueduct or the cisterna magna do not produce pyrexia, (d) injections of 1 μl of a very concentrated dye solution into the putative reactive region give no indication of diffusion to the AH/PO region. However, to prove beyond doubt that transport to the AH/PO region is not responsible for the effect, we feel it is necessary to show that injections into the putative reactive locus are effective in rats in which the AH/PO region has been destroyed bilaterally. We have developed a method for producing such a lesion reproducibly and have shown that lesioned rats are unresponsive to PGE_1 injected into the third ventricle or into the subarachnoid space beneath the lesioned area. At this writing, we are preparing to test injections of PGE_1 into the putative reactive region in rats with AH/PO lesions.

D. Evaluation of the pyrogenic effect of leukotrienes C_4 , D_4 and E_4 (Paper #14).

These experiments dealt with the possibility that arachidonic acid metabolites other than prostaglandins might be pyrogenic. The metabolites of interest to us are leukotrienes C_4 , D_4 and E_4 . These are recently identified arachidonic acid products which arise via the lipoxygenase pathway rather than the more familiar cyclo-oxygenase pathway. They exhibit numerous biological activities and, like the prostaglandins, are very likely involved in inflammation. None of the lipoxygenase products have been examined for pyrogenic activity, perhaps in part because traditional antipyretics have been thought to be specific inhibitors of the cyclo-oxygenase enzyme. However, recent reports that aspirin and indomethacin can inhibit some lipoxygenases suggest that antipyretics may not be as selective for the cyclo-oxygenase pathway as had been believed. Thus, a direct action of lipoxygenase products in fever production is a possibility. Furthermore, some lipoxygenase products can enhance the generation of prostaglandins and other cyclo-oxygenase products and might thus be involved in fever generation through this indirect mechanism. In view of this information, it seemed that an examination of lipoxygenase products for pyrogenic activity would be worthwhile.

The details of the experiments are presented in Paper #14. In summary, we injected 1 μ g doses of leukotriene C_4 , D_4 and E_4 into the third cerebral ventricle of rats which had been shown to develop pyrexia following similar injections of 1 μ g of PGE_1 . We also examined the effect of bilateral injections of 400 ng of each leukotriene into the AH/PO region in rats in which bilateral injections of 40 ng of PGE_1 had been shown to produce pyrexia. None of the leukotriene injections produced a significant increase in body temperature. Dye studies demonstrated that the injection cannulae had been placed correctly. At the completion of the study, the leukotriene samples used were assayed for biological activity using the guinea pig tracheal strip method. It was found that the samples were still highly active. We conclude that the leukotrienes tested are not likely to be involved in the production of fever by cerebral trauma or, for that matter, in any other type of fever.

E. Involvement of supraspinal and spinal neurotransmitters in thermoregulation and fever.

(1) Supraspinal neurotransmitters (Papers #2 and #5).

An oft cited model of thermoregulation devised by R. D. Myers indicates that serotonergic synapses in the rostral hypothalamus are involved in the mediation of the thermogenic and heat re-tentive activity initiated by pyrogens. These conclusions were based on microinjection studies in monkey and cat in which 5-HT injected into the AH/PO region caused very long lasting hyperthermias. These long lasting effects were, we felt, incompatible with the known rapid disposition of 5-HT in brain tissue. We also noted that some workers had reported that intraventricular injections of 5-HT in the cat produced a fall rather than a rise in body temperature. Further, no one had demonstrated that the 5-HT-induced temperature rise reported by Myers was mediated by 5-HT acting on specific 5-HT

receptors. We therefore carried out a study in cats in which 5-HT was injected in various doses into numerous sites within the AH/PO region. The responses were noted, and the experiments were then repeated after systemic injection of indomethacin or after systemic administration or injection into the 5-HT microinjection sites of the 5-HT antagonist, methysergide. The results showed that (a) 5-HT injections into the anterior hypothalamus produced short lasting initial temperature rises which were often followed by prolonged secondary hyperthermias, (b) injections of 5-HT into preoptic sites produced short lasting temperature falls which were often followed by prolonged hyperthermias, (c) both the initial rise and the initial fall produced by 5-HT could be prevented by systemically injected methysergide or by methysergide injected into the 5-HT microinjection site, (d) all of the delayed, prolonged hyperthermias were blocked by indomethacin pretreatment, whereas the initial, short lasting temperature changes were unaffected. These findings indicate that the initial, short lasting falls and rises were produced by 5-HT acting on specific 5-HT receptors and thus presumably reflect a neurotransmitter action of 5-HT in the AH/PO. The delayed and long lasting rises, however, were probably due to prostaglandin release. The data support Myers' suggestion that 5-HT synapses in the anterior hypothalamus could be involved in the mediation of pyrogen induced heat gain and heat retention. However, they also indicate that the 5-HT synapses in the preoptic region could well be involved in heat dissipation rather than in heat gain. It might seem reasonable to suggest that 5-HT released from brain tissue as a consequence of cerebral trauma or 5-HT released from platelets in cases of intraventricular bleeding could act at the anterior hypothalamic synapses involved in heat gain to produce pyrexia. However, our earlier mentioned work shows that indomethacin blocks pyrexias produced by unilateral AH/PO trauma or by intraventricular injection of blood. It is thus unlikely that an action of 5-HT on specific 5-HT receptors is involved in the mediation of these pyrexias. It remains possible, however, that 5-HT released by tissue damage or ventricular bleeding causes brain tissue to release prostaglandins which would, in turn, produce pyrexia. Thus, an involvement of 5-HT in trauma-induced fevers cannot be completely discounted.

The previously mentioned model of thermoregulation adduced by R. D. Myers indicates that, in addition to 5-HT synapses, there are cholinergic synapses in the AH/PO region which are involved in the activation of heat gain effectors. This aspect of the model was based on microinjection studies in the monkey in which cholinergic agonists were injected into the AH/PO region and were found to produce a temperature rise. We carried out similar studies using the cholinergic agonist, carbamylcholine (CCh) and were able to reproduce Myers' results (i.e., core temperature increased). However, we found that the rise was not due to unregulated thermogenesis. Instead, CCh evoked a well regulated temperature increase that was functionally equivalent to a true fever. Thus, cholinergic synapses in the AH/PO region may not be involved exclusively in heat gain. Rather, these synapses seem to be part of the integration system responsible for generation of the setpoint for thermoregulation.

Of more specific relevance to this contract is our additional finding that the hyperthermia produced by CCh was completely prevented by systemic injection of the muscarinic cholinergic antagonist, atropine, whereas sodium salicylate (900 mg p.o.) or indomethacin (10 mg/kg i.m.) had no effect on the pyrexia. This indicates that the CCh effect was caused by an action on AH/PO muscarinic receptors and that prostaglandin release was not at all involved in the response. Therefore, since indomethacin blocks trauma-induced fevers in both of our experimental models, it does not seem likely that acetylcholine released from injured tissue or by some substance present in intraventricular blood can be importantly involved in the pyrexias associated with these models.

(2) Spinal neurotransmitters (Papers #11, #12 and #13).

The final series of experiments supported by the contract was designed to evaluate the roles of spinal neurotransmitters in thermoregulation, with the notion that their involvement in fever production would be studied later. The contract was terminated before these latter studies could be carried out. At the time these studies were undertaken, the role of spinal neurotransmitters in temperature regulation was totally unexplored. It was thought that these neurotransmitters must be of importance because the cord is involved in the upward transfer of sensory information from peripheral and deep body thermodetectors and the downward transfer of impulses controlling the level of activity of thermoregulatory effectors. In addition, the cord itself possesses a population of thermodetector units whose thermoregulatory "potency" is equivalent to that of the thermodetectors in the AH/PO region. The activity of these spinal detectors could well be modulated by serotonergic and/or noradrenergic fibers descending from the brainstem or by cholinergic units intrinsic to the cord.

The reader is referred to the two major publication resulting from these studies (Papers #12 and #13) for details of the methods and results. In brief overview, it was found that injection of norepinephrine (NE), 5-HT and CCh into the lumbar spinal subarachnoid space of rats produced dose dependent thermoregulatory effects. NE produced a transient rise followed by a prolonged fall, 5-HT and CCh produced hyperthermia only. Studies relating to the site and mechanism of action of NE in producing the biphasic core temperature change indicated that the transient rise was probably due to leakage of NE from the subarachnoid space into the general circulation, with subsequent stimulation of heat production by activation of peripheral non-shivering thermogenic mechanisms. The prolonged temperature fall produced by NE was shown to be a direct spinal effect of the drug. Further studies revealed that the fall was probably due to an inhibitory effect of NE on sympathetic preganglionic neurons located in the intermediolateral cell column of the cord, with a resultant inhibition of peripheral vasoconstriction and non-shivering thermogenesis. The hyperthermic effects produced by 5-HT and CCh were also demonstrated to be consequences of direct actions on the spinal cord, but studies of the sites of action and the mechanisms of action have not been carried out.

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